REMARKS/ARGUMENTS

Applicant confirms its **election** filed 09/07/2004 of invention I, claims 1-5, drawn to a vaccine, and the species PlpE protein of SEQ ID NO: 2, in response to the restriction and the species election requirement mailed 08/03/2004. Applicant believes the non-elected claims to be in condition for rejoinder once an elected claim is found allowable.

The original **oath/declaration** was considered defective because of certain non-initialed and/or non-dated alterations appearing therein. A new Declaration is attached.

The **informalities noted in the specification** have been corrected. In particular, paragraph **[0020]** has been amended to refer to --Figures 4A to 4C--. In addition, the various paragraphs of the specification and the drawing figures containing trademarks have been amended, where necessary, to include proper capitalization. It is noted that where names are used as a corporate identifier rather than in a trademark sense (such as in numerous parentheticals), such corporate names have not been capitalized.

The disclosure noted by the Examiner in line 11 on page 26 of the specification has been amended to clarify that the noted recitation is not in fact an amino acid sequence, but rather constitutes part of Applicant's narrative discussing the similarities in the first four (4) residues of various noted sequences. Commas have been inserted between the recited residues to emphasize

the point. In addition, the noted recitation, as amended, now refers to only four (4) amino acids. Thus, no amendment to the original sequence listing is required.

The claims have been amended to address the Examiner's **rejections under 35 U.S.C. §112, 2nd paragraph.** In particular, claims 1 and 3 have been amended to replace "*M.*" with the expanded term --*Mannheimia*--. In addition, claims 3, 4 and 5 have been amended to change the recitation "polypeptide of SEQ. ID NO: 2" to --amino acid sequence of SEQ. ID NO: 2--.

Substantively, the Examiner rejected claims 1-3 under **35 U.S.C. §102(b)** as being anticipated by Pandher et al. (*Infect. Immun.* 66: 5613-5619, December 1998) as evidenced by Hunter (U.S. Patent No. 5,554,372) or Berinstein et al. (U.S. Publication No. 20040033234). The Examiner states in part:

> Pandher et al. taught a composition comprising PBS and a recombinant PlpE outer membrane protein of *P. haemolytica* comprising the amino acid sequence of SEQ ID NO: 2. The protein is expressed via a recombinant *E. coli* (see abstract, Materials and Methods, Figure 1 and 2; and Results). The PlpE is taught to be immunogenic in cattle (see abstract).

The rejection is respectively traversed, as Applicant believes that the Examiner has misapprehended the teachings of Pandher et al. as explained in detail below.

As an initial but important point, it should be recognized that the term "recombinant" is loosely used in Pandher et al.'s (1998)'s paper. In its classical usage, the word "recombinant" is

Amendments to the Drawings:

The four (4) attached replacement sheets of drawings include changes to Figs. 2-5. The changes merely reflect the requested capitalization of trademarks.

Attachments: Replacement Sheets (4)

Annotated Sheets Showing Changes (4)

used to describe either a plasmid DNA construct carrying a gene of interest and hence recombinant DNA or a protein that is expressed and purified from an appropriate expression host, thus the designation recombinant protein. Unfortunately, Pandher et al. use the phrase "recombinant *E. coli*" several times when they might better have said an *E. coli* host expressing PlpE on its surface as an integral part of its outer membrane. Pandher et al. use such an *E. coli* to **absorb antibodies** specific to PlpE from a convalescent serum obtained from a calf with *Mannheimia haemolytica* or serum generated by immunizing animals with outer membrane proteins of the same organism to a state where complement-mediated cell killing activity was reduced significantly. They did not produce recombinant PlpE in its pure form, and did not immunize calves with it. They merely showed that cattle that had recovered from previous *M. haemolytica*-induced disease or ones that had been experimentally vaccinated with the **entire outer membrane** from the bacterium developed antibodies to PlpE. They used the PlpE expressed on the surface of *E. coli* to purify those antibodies and show that they could kill the bacterium in the presence of complement. **Calves were never vaccinated with PlpE or challenged with *M. haemolytica* to demonstrate that this protein had potential vaccine properties.**

In the present case, recombinant PlpE was expressed in *E. coli* BL21 (DE3) pLysS and purified on a nickel affinity column and used to vaccinate calves. The response of the animals was determined by measuring circulating anti-PlpE antibodies on ELISA and Western blots in which purified recombinant PlpE was used as ligand. The protective nature of the specific immune response was demonstrated by challenging the calves with live homologous *M. haemolytica* strain

and bactericidal activity of an anti-PlpE hyperimmune serum in the presence of a complement. Applicants demonstrated directly the immunogenic nature of recombinant PlpE and its potential as vaccine or component of a commercial vaccine. Thus, recombinant PlpE is used herein in the conventional sense, referring to purified PlpE from the *M. haemolytica plpE* gene over-expressed in the expression host and purified.

Pandher et al. (1998) can be considered nothing more than an invitation to try. The antibodies against PlpE that were used by Pandher et al. (1998) were those that were affinity purified from a calf's serum. That calf had been vaccinated with the entire outer membrane of *M. haemolytica* (*M. haemolytica* was called *Pasteurella haemolytica* at that time), which contains at least 21 different immunogenic outer membrane proteins - of which PlpE is only one (Pandher K, Murphy GL, Confer AW., Identification of immunogenic, surface-exposed outer membrane proteins of *Pasteurella haemolytica* serotype 1, *Veterinary Microbiology 65*: 215 – 226, 1999, previously submitted under IDS of July 7, 2004). Pandher et al. (1998) used that serum in an *in vitro* complement-mediated killing assay before and after antibodies to PlpE were removed by adsorption to PlpE expressed on the surface of *E. coli.* They showed that removal of the antibodies to PlpE eliminated complement-mediated killing of *M. haemolytica* and in their discussion state "[r]esults of the complement-mediated killing assays demonstrate that anti-PlpE contribute to this mechanism of bovine defense, one that is believed to be important in protection against *P. haemolytica.*" Therefore, Pandher et al. (1998) demonstrated only indirectly and by *in vitro* laboratory test that there was a potential for antibodies to *M. haemolytica* to be protective

against the bacterium. Again, they did not vaccinate cattle and demonstrate directly that PlpE induced protection. In fact, other potential immune mechanisms that occur in cattle when exposed to a pathogenic agent, like *M. haemolytica*, were not investigated. These include: cell-mediated cytotoxicity; opsonization, phagocytosis and killing; antibody-dependent cytotoxicity; and activation of natural killer cells. Thus, only one of several important mechanisms of host defense was addressed in a single *in vitro* experiment leaving a reader with the question of how relevant are these data to protection of cattle from *M. haemolytica* infection.

In addition, in the Discussion section of Pandher et al. (1998), the DNA sequence identities and similarities between PlpE and *Actinobacillus pleuropneumoniae* OM1A serotypes 1 and 5 are compared. Even though there are similarities between sequences from *M. haemolytica* and *A. pleuropneumonia,* those similarities were not great and consist of only 18 – 20% identity and 32 – 35% similarity between PlpE and Om1A from *A. pleuropneumonia* serotypes 1 and 5. They further described that in vaccination experiments conducted by others with recombinant *A. pleuropneumoniae* Om1A the recombinant protein "...significantly reduced lung damage and death of pigs upon subsequent experimental challenge." They then commented that "...PlpE may have potential for being a significant cross-protective antigen..." and that "Future studies will be necessary to evaluate the capacity of PlpE to enhance protection of cattle against experimental challenge." Those statements were all that were made theoretically linking PlpE with a vaccine. Pandher et al. did not use recombinant PlpE as a vaccine in any form and only showed indirectly that it had any vaccine potential through *in vitro*

complement-mediated killing and inference from publications from a related bacterium – *A. pleuropneumoniae*. Pandher et al. also makes no mention of the potential use of PlpE as an addition to an existing *M. haemolytica* vaccine. Consequently, Pandher et al. cannot be said to anticipate Applicant's claimed invention.

* * * * *

For at least the foregoing reasons, Applicant believes the application to be in condition for allowance, which is respectfully requested.

This paper is intended to constitute a complete response to the outstanding Office Action. Please contact the undersigned if it appears that a portion of this response is missing or if there remain any additional matters to resolve. If the Examiner feels that processing of the application can be expedited in any respect by a personal conference, please consider this an invitation to contact the undersigned by phone.

Respectfully submitted,

3/30/05

DATE

SIGNATURE OF PRACTITIONER

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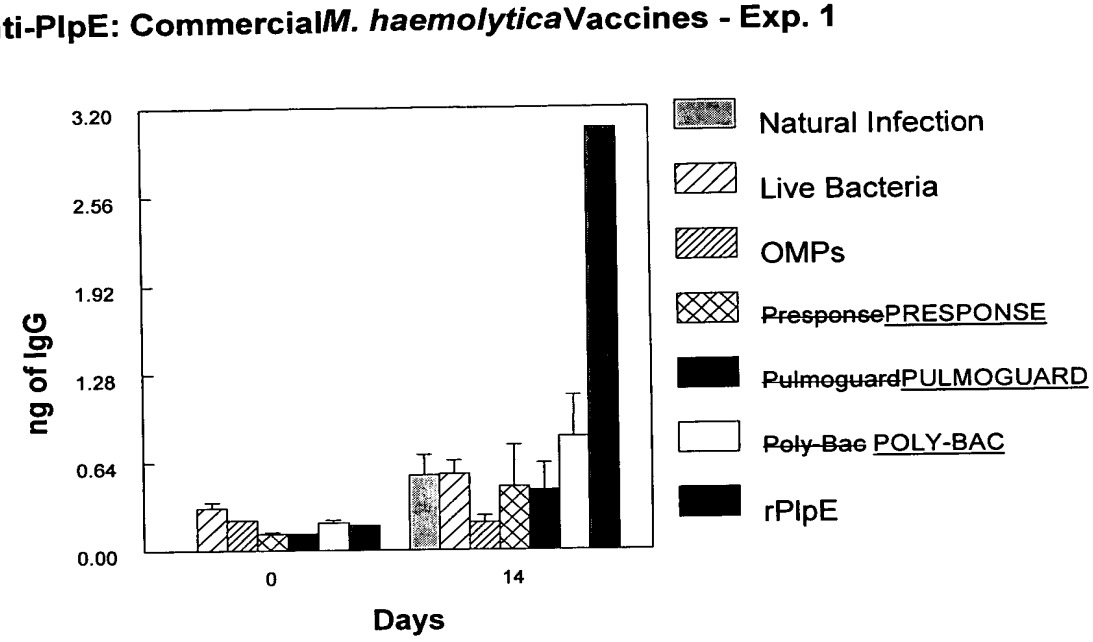

FIG. 2

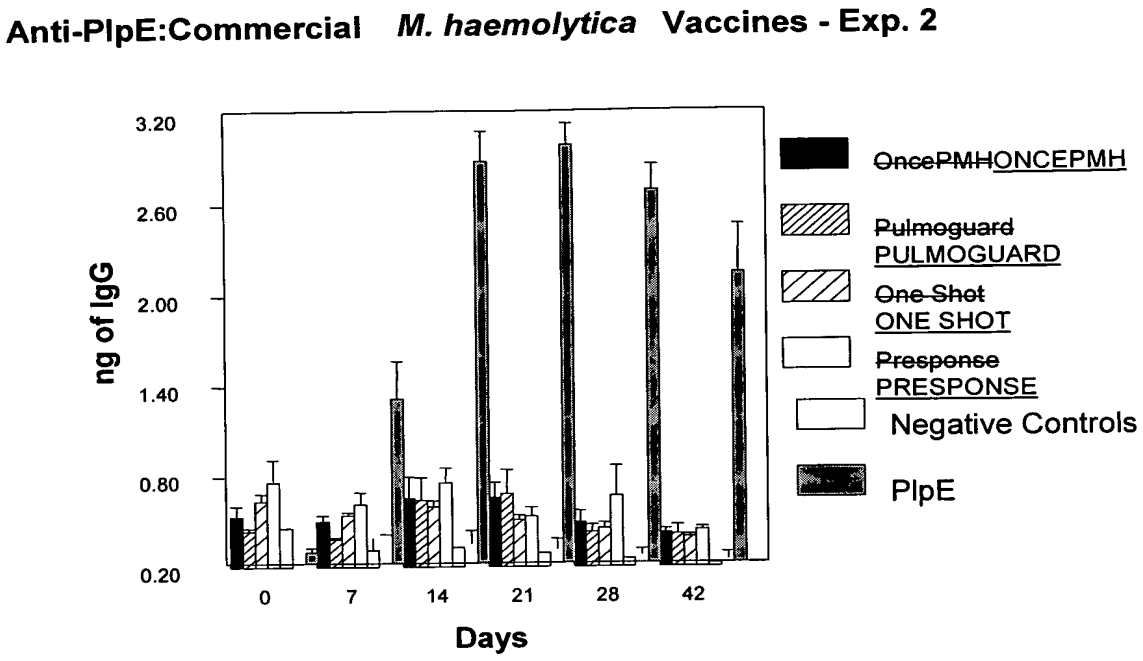
Anti-PlpE:Commercial M. haemolytica Vaccines - Exp. 2
ng of IgG
3.20
2.60
2.00
1.40
0.80
0.20
0
7
14
21
28
42
Days
~~OncePMH~~ONCEPMH
~~Pulmoguard~~ PULMOGUARD
~~One Shot~~ ONE SHOT
~~Presponse~~ PRESPONSE
Negative Controls
PlpE

FIG. 3

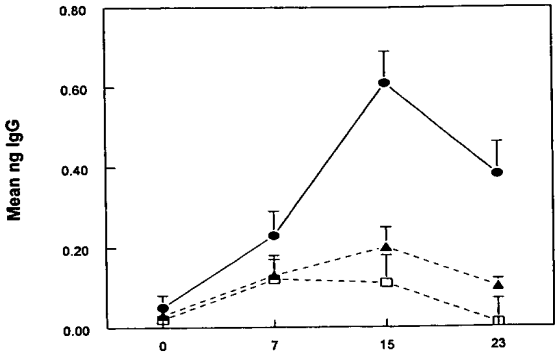

FIG. 4A

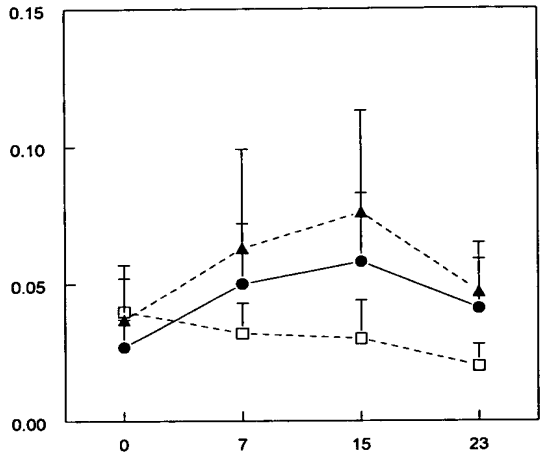

FIG. 4B

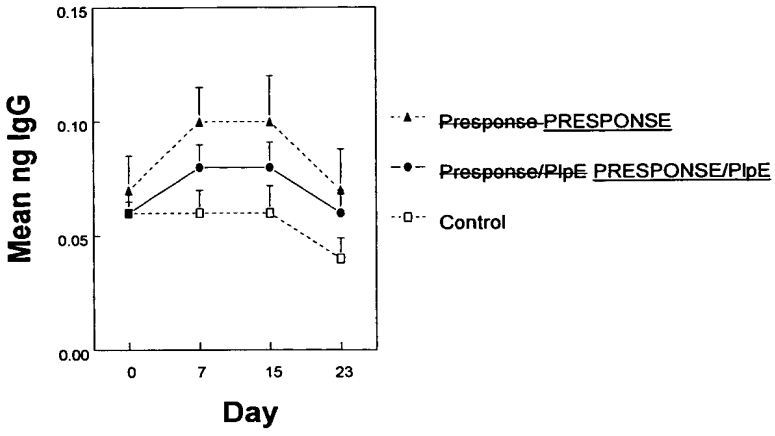

FIG. 4C

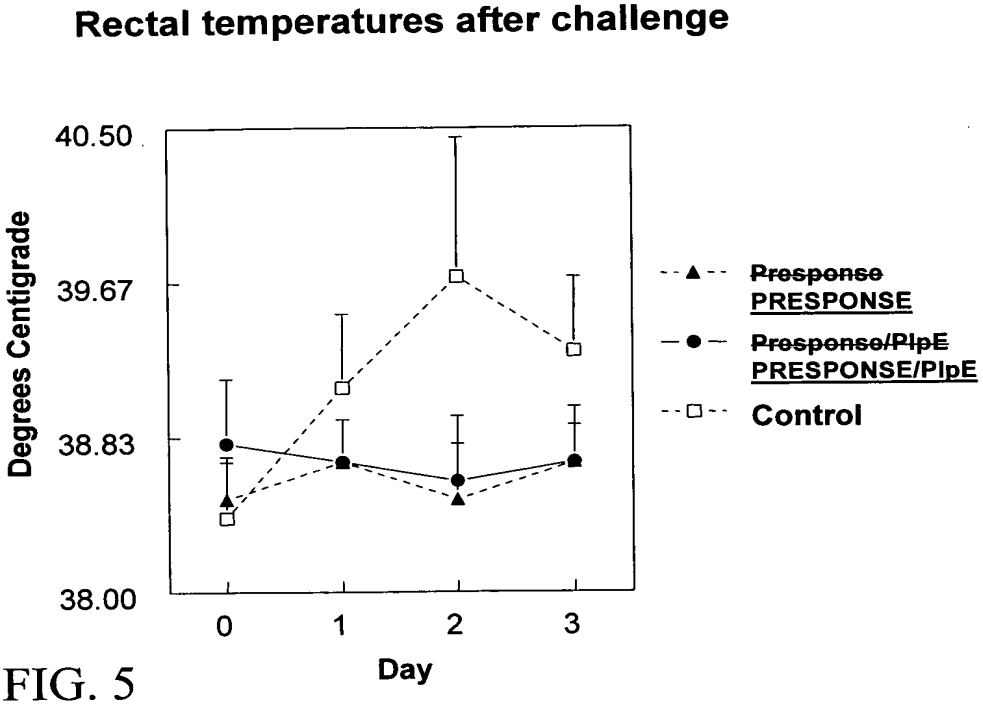
Rectal temperatures after challenge
40.50
39.67
38.83
38.00
Degrees Centigrade
0
1
2
3
Day
~~Presponse~~ PRESPONSE
~~Presponse/PIpE~~ PRESPONSE/PIpE
Control

FIG. 5